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LETTERS AND CORRESPONDENCE

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Fludarabine-Related Hemolytic Anemia in Chronic Lymphocytic Leukemia and Lymphoproliferative Disorders

To the Editor: Fludarabine, which is a potent inhibitor of adenosine deaminase, is an effective agent in the treatment of chronic lymphocytic leukemia (CLL) and low-grade non-Hodgkin's lymphoma (NHL) [1]. One of its major side effects is a profound T-cell immunosuppression and autoimmune hemolytic anemia (AIHA) [2]. To investigate the incidence and any risk factors of development of AIHA, we conducted a retrospective study of 20 patients with advanced CLL and low-grade NHL resistant to treatment with alkylating agents, who were treated with fludarabine between 1994–1996.

Four patients with CLL and small cell cleaved NHL with a mean age of 64 years (range, 45–72 years) developed AIHA after treatment with fludarabine. All had stage C disease for the three cases of CLL, and stage III B for the case with NHL. Three patients had no previous history of hemolysis and a negative Coombs' test before the start of fludarabine treatment. One patient had a history of AIHA that was controlled with low-dose prednisone at the time of treatment with fludarabine and Coombs' test reverted to negative. All developed severe AIHA with positive Coombs' test after a median of four courses (range, 2–6 courses). All patients required treatment with prednisone and in some cases red cell transfusion. None of these patients had a recurrence of the hemolytic reaction after tapering of steroids but only one patient had a negative Coombs' test after treatment. None of these patients restarted on fludarabine after control of hemolysis.

The incidence of AIHA in patients treated with fludarabine was investigated by Di Raimondo et al. [3]. They found five patients without pre-existing AIHA and four patients with pre-existing AIHA who developed hemolysis after one to six courses of fludarabine among a group of 112 patients with CLL (8%); however, others reported a higher incidence (21%) [4]. In most cases, hemolysis occurs after a mean of four courses of fludarabine and tends to be severe and has an abrupt onset. Usually hemolysis can be controlled by prednisone at a dose of 1 mg/kg. In some cases it is possible to administer further courses of fludarabine safely. But, commonly, further courses of fludarabine lead to hemolytic exacerbation that is usually difficult to control. In our study, we have a patient with a pre-existing AIHA who developed hemolysis after only two courses of treatment, which is consistent with the possibility that patients with pre-existing AIHA are at a higher risk of developing this complication. It is recommended that such patients either receive other forms of treatment or receive fludarabine with prophylactic corticosteroids because patients receiving a combination of fludarabine and corticosteroids are at a higher risk of systemic infection. It is recommended that this treatment be given under both antifungal and antipneumocystis protection. The mechanism of fludarabine-related AIHA is not clear. It may be related to the tendency of fludarabine to produce profound and long-lasting T lymphopenia. Self-tolerance is believed to be maintained by the suppression of autoreactive T cells by autoregulatory T cells. It seems that fludarabine enhances the T-cell defect and greatly increases the risk of autoimmunity [5].

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Prolymphocytic Transformation of B-Chronic Lymphocytic Leukemia Presenting as Malignant Ascites and Pleural Effusion

Prolymphocytic transformation is a complication of chronic lymphocytic leukemia (CLL) associated with increasing splenomegaly, leukocytosis

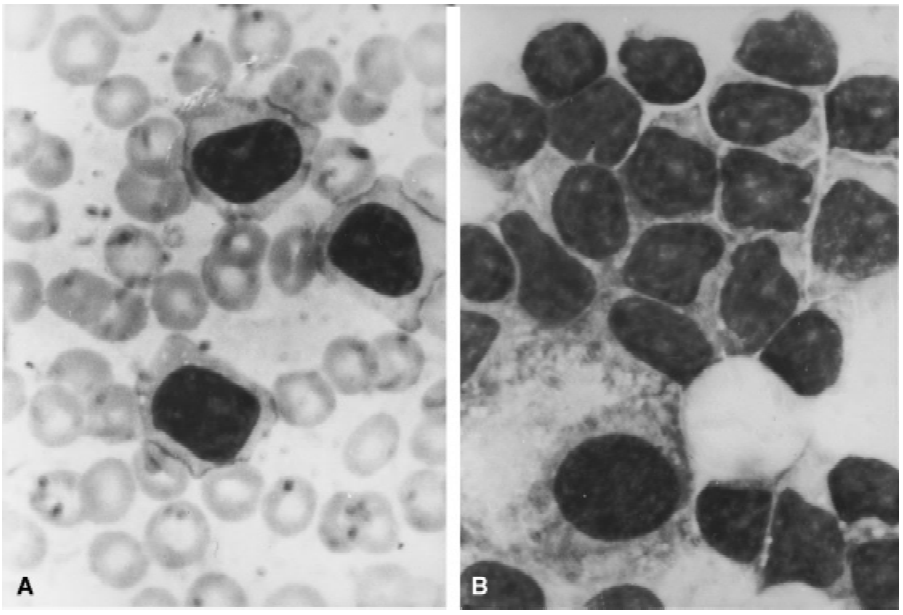

Fig. 1. A: Peripheral blood smear. MCG staining (×800). Prolymphocytes. B: Cytospined smear of ascitic fluid. MCG staining (×800). One mesothelial cell and prolymphocytes.

with more than 55% prolymphocytes, and resistance to therapy. We herein report a patient with unusual development of ascites and pleural effusion at presentation of the prolymphocytic transformation.

An 81-year-old woman presented with dyspnea and anasarca developing gradually over two months. She was diagnosed 10 years earlier as Rai stage 0 CLL following routine blood count, but was asymptomatic and did not have hematological follow-up since then. Physical examination revealed tachypnea, right pleural effusion, tense ascites, and 15 cm splenomegaly. No lymph nodes could be palpated. Complete blood count revealed hemoglobin, 12.3 g/dl; white blood count 80×10^9/l (80% prolymphocytes [Fig. 1A]); and platelets, 91×10^9/l. Serum biochemistry was normal. Serum immunoglobulins (IG) were IgG, 499 mg/dl (normal, 1,020–1,460); IgM, 1,150 (normal, 85–155); IgA, 259 (normal, 210–350). A paraprotein IgM kappa was found on electrophoresis and immunofixation. Chest X-ray revealed a right pleural effusion. Abdominal ultrasound showed an enlarged spleen (21 cm) and liver (18 cm), ascites, normal venous flow, and no lymphadenopathies.

Prolymphocytes expressed HLA-DR, CD19, FMC7, and were CD5-. A diagnosis of prolymphocytic transformation of CLL was established. Two liters of pleural and one liter of ascitic fluid were removed for symptomatic relief. Ascitic fluid was an exudate with a low albumin gradient (2.7 g/dl in ascites and 3.6 in serum). Cultures for bacteria and mycobacteria were negative. Cytospin analysis showed 20×10^9/l cells, mainly prolymphocytes (Fig. 1B) similar to those found in PB with the same immunophenotype. Pleural fluid had the same characteristics. The patient was treated by COP (cyclophosphamide, vincristine, and prednisone) with partial response and then by six courses of mini-CHOP (same, with addition of low dose doxorubicin). At the end of treatment the spleen was palpated three cm below costal margin; hemoglobin, 11.6 g/dl; WBC, 10.4×10^9/l (40% prolymphocytes); and platelets 120×10^9/l. IgG rose to 810 and IgM decreased to 430. A good response was therefore achieved.

Prolymphocytic leukemia (PLL), characterized by hyperleukocytosis, massive splenomegaly, minimal lymphadenopathy, and large lymphocytes with immature-looking nucleus seen on PB smears, may occur de novo or as transformation from CLL. The patient who presented had CLL for many years, was asymptomatic, and did not require treatment. On presentation she had morphological and immunological criteria for the diagnosis of PLL [1]. Only few cases of ascites in CLL have been reported. In two cases it was caused by portal hypertension due to infiltration of hepatic portal spaces by leukemia or thrombosis of the portal vein [2,3]. Another two cases of Richter's transformation presenting as ascites [4,5] and one of chylous ascites have been reported [6]. Pleural effusion, although rare in CLL, is more common than ascites. The differential diagnosis of ascites can be facilitated by the determination of serum-ascites gradient [7]. A low gradient <1.1 or exudative ascites is typical for infection or malignancy, whereas portal hypertension causes ascites with a high gradient. Our patient had a low gradient therefore excluding portal hypertension as the cause of ascites. The fluid was sterile. Most of the cells were prolymphocytes with the same morphology and immunophenotype as those found in the blood. The pleural effusion was an exudate with the same characteristics. The patient responded dramatically to chemotherapy. All these suggest that ascites and pleural effusion in our patient were due to PLL infiltration of the peritoneum and pleura and were true malignant effusions resembling that found in solid tumors.

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BRAC2 Gene Deletion Is Rare in Chronic Lymphocytic Leukemia

To the Editor: It was recently reported by Garcia-Marco et al. [1] that the BRCA2 gene is deleted in the lymphocytes of the majority of patients with chronic lymphocytic leukemia (CLL). The authors demonstrated the deletion of the 13q12 locus encompassing the BRCA2 gene in their patients using interphase cytogenetic analysis. However, in a subsequent study, Panayiotidis et al. [2] reported no deletion of the BRCA2 gene in any of 24 CLL DNA samples investigated by Southern blotting.

We have carried out a further study in 34 CLL patients to investigate this important question. We performed gene dosage analysis using Southern blotting to determine whether the BRCA2 gene is deleted in CLL. DNA was extracted from the lymphocyte cell population of patients with CLL and from peripheral blood leukocytes obtained from a number of healthy controls. Patient and control DNA samples were digested with *Eco*RI and size fractionated through a 1% agarose gel. Southern blotting was performed according to standard procedures. Filters were hybridized to the probe D13S25 to detect the 13q14 deletion described previously in CLL [3–5] and polymerase chain reaction (PCR) generated probes for exon 11 and exons 26–27 of the BRCA2 gene. The renin probe (mapping to the chromosome 1q42) acts as an internal hybridization standard and was used as the control probe for densitometry. After autoradiography the film was scanned by a computerized densitometry software to quantitate the relative intensities of the hybridization signals. Our results are shown in Table I.

Frequent loss of the D13S25 marker has been reported in CLL. We found the percentage of patients with heterozygous and homozygous loss with the D13S25 probe to be very similar to the results reported previously. In our series only two cases out of 34 showed heterozygous loss of the BRCA2 gene and essentially our results are in agreement with those of Panayiotidis et al. [2]. We conclude that loss of BRCA2 is rare in CLL.

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TABLE I. Summary of the Results of Cases Investigated for Loss of the D13S25 and BRCA2 Loci

Genomic status	D13S25	BRCA2 exon 11	BRCA2 exon 26–27
No loss	16	32	32
Heterozygous loss	13	2[a]	2[a]
Homozygous loss	5	0	0
Total cases	34	34	34

[a]The two cases showing the loss with the BRCA2 exon 11 probe are the same cases showing loss with BRCA2 exon 26–27.

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Transient Megakaryoblastic Feature in a Patient With Diamond-Blackfan Anemia

To the Editor: Transient myeloproliferative disorder (TMD) is almost always coexistent with Down's syndrome [1]. It rarely progresses and necessitates therapy [1]. Diamond-Blackfan anemia (DBA) is an inherited disease which may present with physical abnormalities.

We report a case of DBA with megakaryoblastic myeloproliferation, as the first case of DBA with myeloproliferative syndrome.

The patient was born after a 36.5 weeks' of gestation with low birth weight. He had cleft palate, inguinal hernia, and partial agenesis of musculus orbicularis oris. His blood chemistry, urine and blood amino acids, echocardiography, and cranial computed tomography were all normal. On the 41st day of life, he was transferred to the Hematology Department because of anemia that had developed gradually (Table I). On the blood smear, macrocytosis and lymphocyte-like cells producing platelets were detected and CD41 was 71% positive by flow cytometry. The bone marrow aspirate was normocellular, megakaryoblasts and normoblasts were 45% and 3%, respectively. CD41 was also positive by 53%. Cytogenetic analysis showed 46 XY with few nonspecific chromosomal breaks. In differential diagnosis, TMD and possible DBA were considered. He required erythrocyte suspensions regularly. Megakaryoblasts on the blood smear and bone marrow with normoblastopenia were found to persist when he was seven months old. During the follow-up, the patient persisted to display the findings of isolated DBA but no megakaryoblastosis. High-dose methyl prednisolone was started on the 16th month of age (Table I). On the 28th day of the treatment hemoglobin (Hb) was 11.6 g/dl. His Hb level changed between 6–10 g/dl without transfusion. Now his Hb is 10.6 g/dl and he is on methyl prednisolone therapy of 0.3–1 mg/kg/day.

When he was three years old, his weight and height were below the third percentile. The mean corpuscular volume (MCV) was 103 fl. Hb F was 7%, adenosine deaminase (ADA) in erythrocytes 74.03 (N: 40–60) μmol uric acid/hr/gm Hb, and ferritin 541 ng/ml.

The patient who had physical abnormalities and the aforementioned laboratory findings was considered as ''TMD with transient megakaryoblastic myeloproliferation.''

Recent reports describing the immunophenotyping findings suggest that blast cells in acute myeloid leukemia (AML) M7 and TMD are consistent with undifferentiated progenitor cells around the level of colony-forming unit-granulocyte/erythroid/monocyte/magakaryocyte (CFU-GEMM) [2]. Some other investigators suggest that erythroid and megakaryocytic differentiation pathways are closely related to each other and progenitor cells common to these two lineages may exist [3]. It was shown that the tran-